(12) # EUROPEAN PATENT APPLICATION

---

(71) Applicant: DAMON BIOTECH, INC., 119 fourth Avenue, Needam Heights Massachusetts 02194 (US)

(72) Inventor: Lim, Franklin, 6500 Boatwright Drive Richmond, Virginia 23236 (US)

(74) Representative: Blatchford, William Michael, Withers & Rogers 4 Dyer's Buildings Holborn, London EC1N 2JT (GB)

(54) Sustained release capsule and process of making it.

(57) A dispensing system for the sustained releasing of a substance at a substantially constant rate includes semipermeable capsules containing a material to be released. The capsules comprise membranes having pores of dimensions sufficient to control the kinetics of release provided there is maintained a large intracapsular concentration of the substance relative to the concentration desired in the extracapsular environment. The system may be produced by forming permeable capsules, suspending the capsules in a solution containing a high concentration of the substance to load the intracapsular volume, and then post-treating the capsules to reduce the size of the pores.

EP 0 126 537 A2

0126537

SUSTAINED RELEASE

This invention relates to microencapsulation. More particularly, it relates to a method of loading permeable microcapsules and a composition of matter comprising a

5   permeable microcapsule containing one or more substances which may be released from the intracapsular volume at a substantially constant rate for a significant time.

Compositions of matter capable of sustained release of drugs, fertilizers, and the like are well known in the

10   art. Generally, such compositions comprise a solid carrier in which the substance to be released is absorbed or trapped as plural separate phases. When such compositions are placed in the environment in which they are intended for use, the outer layers of the solid

15   carrier dissolve releasing a portion of the substance of interest. If the solid carrier dissolves slowly, then the substance of interest is released slowly or in discrete bursts over time. In use, the rate of release of the substance from such compositions necessarily is dependent

20   upon the solubility of the carrier particle material, on the surface area of the carrier particle that at any given time is exposed to the environment, and often on the solubility of the substance in the environment of use. These factors typically result in a release rate which

25   decreases over time.

In situations where it is desired, e.g., to release a drug at a constant rate into the circulatory system, the foregoing approach based on solubility properties cannot be used. In such situations, implantable

30   mechanical perfusion pumps and similar devices have

been suggested.

It has now been discovered that sustained release at a substantially constant rate can be achieved for essentially any substance by providing a solid or liquid
5   reservoir of the substance within the interior volume of one or more capsules. Each of the capsules comprise a membrane having pores of dimensions sufficient to limit the rate at which molecules of the substance pass there-through. The capsules are used in an environment which
10 depletes the substance, e.g., by chemical modification, sorption, metabolic breakdown, ingestion, diffusion, or simple removal by fluid flow.

The concept of the invention involves loading permeable capsules with a reservoir of the substance of
15 interest at a concentration sufficient to provide an osmotic pressure above a threshold level, and controlling the pore size of the capsule membranes so that passage of molecules of the substance through the membrane becomes the rate-limiting factor in dispensing the sub-
20 stance into the extracapsular environment. In one important embodiment, the substance of interest is a solid contained within the capsule together with a solvent for the solid. Once the solid and its solvent reach equilibrium, a reservoir of molecules of the solvent is
25 available to provide a continuous, substantially constant migration of molecules through the membrane. In another embodiment, the substance is contained within the capsule as a solution at a concentration in excess of the desired extracapsular concentration. Molecules of the substance
30 are released into the extracapsular environment at a

substantially constant rate until the intracapsular concentration drops to a level where the intracapsular osmotic pressure is insufficient to support the membrane dependent transfer rate.

5   The intracapsular concentration of the substance to be dispensed should be quite high, typically at least two orders of magnitude greater than the desired extra-capsular concentration, and more preferably at least three. Generally, the higher the intracapsular concen-
10 tration the longer the constant release rate can be sustained. If the capsules are placed in an environment with no mechanism for removing the substance, then eventually the intracapsular and extracapsular concentration will equalize. Thus, capsules may be stored as a
15 suspension in a volume of a compatible solvent containing a concentration of the substance substantially equal to or greater than the intracapsular concentration. In this circumstance net flow of the substance out of the capsules is prevented.

20   A preferred process for producing the dispensing systems of the invention involves forming capsules having membranes which permit traverse of the substance to be loaded therewithin, suspending the capsules in a concentrated solution of the substance one or more times
25 to diffuse the substance into the intracapsular volume, and thereafter further treating the capsule to reduce the dimensions of the pores in the membrane.

A preferred method of making the capsules involves forming shape-retaining spheres, e.g., on the order of
30 0.1mm to 3mm in diameter from a water-soluble polymer

containing plural anionic or cationic groups and cross-
linking surface layers of the spheres by contact with a
polymer having plural groups of a charge opposite that
of the water-soluble polymer. After loading, the capsule
5   is again treated with the same or a different cross-
linking polymer to reduce the dimensions of the pores.

One outstanding advantage of the process and
dispensing system of the invention is that it can be
modified readily to dispense essentially any substance
10   desired at a controlled, substantially constant rate.
Another advantage is that the capsule membranes can be
fabricated from non-toxic materials that produce no
significant immunological response, and accordingly are
well suited for administration of biologically active
15   materials such as hormones, antibodies, antigens, enzymes,
lymphokines, vaccines, and natural or synthetic drugs via
implantation in an animal body. Additional uses for the
dispensing system of the invention include the sustained
release of fertilizers, pesticides, fungicides, plant
20   hormones and growth factors, flavors, perfumes, preserv-
atives, and nutrients such as tissue culture nutrients.

Accordingly, it is an object of the invention to
provide a novel composition of matter capable of sustained
release of a substance into an environment at a sub-
25   stantially constant rate. Another object is to provide a
method of loading microcapsules. Another object is to
release a biologically active material into an animal body
at a substantially constant rate. Yet another object is
to provide an improved method of controlling the pore
30   size of a microcapsule membrane.

The invention will now be described by way of example with reference to the drawings in which:-

Figure 1 is a graph of measured extracapsular insulin concentration versus time illustrating the substantially constant release rate of insulin using a composition embodying the invention which employs crystalline insulin;

Figure 2 is a graph of the cumulative extracapsular insulin concentration achieved using the preparation of Figure 1;

Figure 3 is a graph of cumulative insulin concentration in an extracapsular medium versus time using a composition of the invention employing a solution of insulin;

Figure 4 is a graph of myoglobin concentration versus time illustrating the substantially constant rate of release of myoglobin; and

Figure 5 is a graph of hemoglobin concentration versus time.

The system of the invention involves the production of spheroidal capsules each of which comprise membranes having pores of dimensions small enough to hinder passage of molecules from a reservoir of a substance within the capsule. The rate at which molecules are released into the extracapsular volume is controlled by the kinetics of their passage through the membrane.

Well established principles of mass transfer across a porous membrane dictate that where the pores of the membrane are sufficiently large, and there is a concentration gradient across the membrane, solute will flow

from the more concentrated region to the less concentrated region at a steadily falling rate until both sides of the membrane are in equilibrium. This equilibrium will also be attained where the pores of the membrane are of

5   dimensions sufficient to hinder passage of mass across the membrane, provided some finite mass transfer occurs. However, in the latter situation, where the interior of a capsule membrane has a high concentration of the solute and the outside is at a low concentration , the passage

10 across the membrane may be the diffusion rate limiting step. Accordingly, until such time that the concentration outside the capsule is high enough to produce a significant balancing osmotic pressure, or such time that the concentration of solute within the capsule becomes

15 insufficient to generate sufficient osmotic pressure to drive the steady diffusion across the membrane, the rate at which solute molecules diffuse out of the capsule will be substantially constant.

The dispensing system of the invention is designed

20 for use where some physical, chemical, or biological mechanism depletes the solute concentration immediately outside the capsule membranes. Accordingly, there is no significant buildup of the extracapsular concentration. Thus, for as long as there is a sufficient concentration

25 of solute within the capsule, the diffusion kinetics are controlled by the capsule membrane. Once an equilibrium outflow of the solute is attained, diffusion proceeds at a substantially constant rate until the intracapsular concentration falls below a level where it influences

30 the diffusion kinetics.

From the foregoing it is apparent that the ability to fabricate capsule membranes having the desired permeability characteristics, and the ability to load the capsules with a high concentration of the substance to be released are both important to the practice of the invention.

Procedures for encapsulation which enable some control of membrane porosity are known in the art, see, for example, U.S. Patent No.4,352,883.   It has been discovered that by suitably modifying the procedures disclosed in the foregoing U.S. patent, the disclosure of which is incorporated herein by reference, it is possible to determine empirically a procedure for producing capsule membranes with the required permeability characteristics.   The principles used to control membrane porosity are disclosed herein.   Furthermore, several procedures for loading the capsules with a high concentration of the substance to be released have also been developed.

In the encapsulation technique of the '883 patent, a water-soluble polycationic or polyanionic polymer is formed into a spherioidal, shape-retaining droplet which suspends the material to be encapsulated.   Thereafter, the droplet is exposed to a polymer having the opposite charge to cross-link surface layers of the droplet by the formation of salt bridges between the polyanionic and polycationic polymers.   This procedure produces a porous membrane.

The material to be encapsulated is prepared in accordance with well-known techniques in finely divided

or solution form, and a water-soluble polyanionic or
polycationic material which can be reversibly gelled is
added to the suspension or solution in low concentration.
The droplet containing the material to be encapsulated is
5   immediately rendered water-insoluble and gelled, at least
in surface layers. Thereafter, the shape-retaining
capsules are provided with a permanent semipermeable
membrane. Where the material used to form the temporary
capsules permits, the capsule interior may be reliquified
10   after formation of the permanent membrane. This is done
by re-establishing the conditions in the medium at which
the material is soluble.

The material used to form the gelled droplets may
be any water-soluble material which, by a change in the
15   surrounding temperature, pH, or ionic environment or
concentration, can be converted to a shape-retaining
mass. The material must also comprise, for purposes of
this invention, plural, easily ionized groups, e.g.
carboxyl or amino groups which can react by salt formation
20   with polymers containing plural groups which ionize to
form species of the opposite charge. Preferred materials
for forming the temporary capsule are water-soluble,
natural or synthetic polysaccharides. Sodium alginate
is most preferred. Other usable polysaccharides include
25   carboxylated fractions of guar gum, gum arabic,
charageenan, pectin, tragacanth gum, or xanthan gum.

These materials comprise glycoside-linked saccharide
chains. Many contain free acid groups, which are often
present in the alkali metal ion form, e.g., sodium form.
30   If a multivalent ion such as calcium or strontium is

exchanged for the alkali metal ion, the liquid, water-
soluble polysaccharide molecules are "cross-linked" to
form a water-insoluble, shape-retaining gel which can be
resolubilized on removal of the ions by ion exchange or
5 via a sequestering agent. While essentially any multi-
valent ion which can form a salt with the saccharides is
operable, physiologically compatible ions, e.g., calcium,
is preferred for purposes of making capsules designed
for the sustained release of drugs. Other polysaccharides
10 can be switched between the water-soluble and gelled,
water-insoluble state simply by changing the pH of the
medium in which they are dissolved.

In the next step of the process, a semipermeable
membrane is deposited about the surface of the gelled
15 droplets. The preferred technique for forming the
membrane is to cross-link surface layers of the droplets
by subjecting them to an aqueous solution of a polymer
containing groups reactive with functionalities in the
gel molecules. Certain long chain quaternary ammonium
20 salts may be used for this purpose in some circumstances.
When acidic gels are used, polymers containing acid
reactive groups such as polyethylenimine, polyvinyl amine,
and polypeptides such as polylysine may be used. In this
situation, the polysaccharides are cross-linked by inter-
25 action between the carboxyl groups and the amine groups.

It has been discovered that permeability can be
controlled sufficiently for purposes of the invention
by selecting the molecular weight of the cross-linking
polymer used, adjusting the concentration of the cross-
30 linking polymer, and by cross-linking in two or more

separate stages using the same or a different cross-
linking agent. Generally, a solution of polymer having
a low molecular weight, in a given time period, will
penetrate further into the temporary capsules than will a
5 high molecular weight polymer.   In general, the higher
the molecular weight and the less penetration, the larger
the pore size. Broadly, polymers within the molecular
weight range of 3,000 to 100,000 daltons or greater may
be used, depending on the duration of the reaction, the
10 concentration of the polymer solution, and the degree of
permeability desired. The currently preferred molecular
weight range is 20,000 - 80,000. Post-treating the
capsule with the same or a different cross-linking
polymer has the effect of reducing the dimensions of
15 the pores and thickening the membrane.

At this point in the encapsulation, capsules may be
collected which comprise a permanent semipermeable
membrane surrounding a gelled solution of polysaccharide
containing the core material. If mass transfer is to be
20 promoted within the capsules and across the membranes,
it is preferred to reliquify the gel to its water-soluble
form. If low molecular weight polysaccharide is used,
much of the excess material can be removed from within
the membrane by diffusion. The liquification may be
25 conducted by re-establishing the conditions under which
the water-soluble polymer is a liquid, e.g., changing
the pH of the medium or removing the calcium of other
multifunctional cations. In the gels which are insoluble
in the presence of multivalent cations, the medium in the
30 capsule can be resolubilized simply by immersing the

capsules in phosphate buffered saline, which contains alkali metal ions and hydrogen ions. Monovalent ions exchange with the calcium or other multifunctional ions when the capsules are immersed in the solution with

5   stirring. Other salts, e.g., sodium citrate, may be used for the same purpose.

Lastly, depending on the type of semipermeable membrane formation technique employed, it is often desirable to treat the capsules so as to tie up free amino

10   groups or the like which would otherwise impart to the capsules a tendency to clump. This can be done, for example, by immersing the capsules in a dilute solution of sodium alginate.

In this encapsulation technique it is difficult to

15   synthesize the membranes while simultaneously retaining a high concentration of the substance to be encapsulated within the forming membrane unless substance has a high molecular weight, i.e., equal or greater than about 300,000.   One solution to this problem is to encap-

20   sulate a solid material. After the capsules are completed, they are placed in the environment of use where solvent dissolves the intracapsular solid. Thus, there is produced within the intracapsular volume a reservoir of solute, at a concentration related to the solubility product of

25   the solid in the solvent. The reservoir is maintained until the solid dissolves.

Another loading technique involves forming permeable capsules which have no initial loading or relatively low loading and thereafter suspending the capsules in a

30   concentrated solution of the substance to be encapsulated.

0126537

Thus, the capsules are loaded from the outside. There-after, the capsules are again treated with a polymeric cross-linking agent of the type described above to reduce further the dimensions of the pores.

5   When employing this outside loading technique, it is preferred that the solution in which the capsules are suspended be as concentrated as possible; at least three orders of magnitude greater than the extracapsular concentration desired during use of the compositions,

10 and preferably even higher. Especially where the substance to be loaded has a high viscosity or a low solubility, it is preferred to repeat the outside loading procedure one or more times. It is also preferred to produce the capsule membrane at the outset so as to

15 provide pores of dimensions sufficient to retard the passage of the solute into the capsule. This lengthens the loading time, but also minimizes leakage of the loaded materials during the time the second cross-linking step is being conducted to further reduce the pore

20 dimensions and thicken the membrane.

From the foregoing it should be apparent that some experimentation will be required in the design of any specific composition for dispensing a given substance. However, in view of this disclosure those skilled in the

25 art will be able to produce a variety of specific compositions having a desired constant rate of release. In some cases it is difficult to set the constant release rate of a given microcapsule at a specific desired level. However, it is relatively simple to

30 control the average rate of release of a large number of

capsules at an arbitrary value. Thus, dosage can be controlled by supplying a number of capsules which together release the desired quantity of the substance to be dispensed at a constant rate over a significant time.

Control of the permeability characteristics of the membrane is exercised by varying 1) the materials used to make the membranes, 2) the polymer concentration of the cross-linking solution, 3) the molecular weight of the cross-linking polymer or polymers, 4) the duration of the cross-linking step, and 5) the number of cross-linking steps conducted. While the effect on permeability characteristics of any one variable isolated from the others is difficult to define precisely, the following general comments can be made. Materials which have a high density of charged groups tend to result in highly cross-linked membranes characterized by smaller pore dimensions. Higher concentrations of polymer and longer exposures to the polymer tend to reduce the dimensions of the pores in the dimensions of the plane of the membrane, and produce thicker membranes with greater pore lengths. Low molecular weight materials tend to penetrate further into the gel. This results in reducing the permeability of the membrane. Cross-linking having a molecular weight below about 3,000 daltons tend to permeate and react with substantially the entire gel sphere. Higher molecular weight materials form a relatively thin membrane on surface layers of the gel. Residual polysaccharide is available to bond with additional quantities of the cross-linker in multiple step cross-linking processes. The use of such multiple

separate cross-linking steps tends to reduce the pore
size.

    The membrane is believed to comprise a matrix of
polymers cross-linked with ionic bonds. The polymers
5 define random intermolecular spaces which communicate
with each other to form tortuous path pores through the
membrane. It is also believed that both the pore
dimensions and the effective length of the pore across
the membrane influence the kinetics of molecular diffusion.
10 Molecules in the volume of the pores presumably undergo
many random collisons which in the aggragate determine
the average time it takes for a molecule of a given
effective dimension to traverse the membrane.

    The substances which may be encapsulated in
15 accordance with the invention to produce compositions
characterized by substantially constant rate, sustained
release can vary widely. The only limiting factors
appear to be that it is difficult to produce a membrane
that will be the dominating factor in controlling the
20 diffusion rate in the case of very low molecular weight
materials, e.g., 200 daltons or below. Also, capsule
membranes uniformly permeable to substances having a
molecular weight greater than about 10^6 daltons are
difficult to synthesize.

25   The invention will be further understood from the
following non-limiting examples, wherein all percentages
are given in a weight/volume basis (g/ml).

        Example 1 - CRYSTALLINE INSULIN CAPSULES

    Gelled spheres were formed from 1.2% sodium
30 alginate (NaG) in 0.9% NaCl containing 20mg/ml (500 units)

crystalline insulin. The NaG-insulin suspension was well mixed and the gell spheres prepared immediately so that the insulin was uniformly distributed in the NaG. Liquid spheroidal drops of the suspension were then immersed in a 1.5% $CaCl_2/H_2O$ gelling solution. After formation, the gelled spheres were allowed to remain in the $CaCl_2$ solution for three minutes and then treated as described below. All treatment and wash solutions had a volume of ten times the volume of the original NaG solution.

1. Treat with 0.6% $CaCl_2$ in 0.45% NaCl for five minutes, maintaining the gelled spheres in suspension.

2. Treat with 0.3% $CaCl_2$ in 0.68% NaCl for five minutes, maintaining the gelled spheres in suspension.

3. Wash twice with saline.

4. Treat with 60,000 molecular weight poly-1-lysine (PLL) in saline (13mg PLL hydrobromide/dl) for five minutes, maintaining the capsules in suspension to form a membrane.

5. Wash twice in saline.

6. Wash with CHES buffer (2% 2(N-cyclohexylamino) ethanesulfonic acid in 0.6% NaCl, pH 8.2) diluted 1:20 with 1.1% $CaCl_2$.

7. Wash twice with saline.

8. Treat with 0.11% polyvinylamine (PVA Polyscience), molecular weight range 50,000 to 150,000 in saline, pH 7.0, for five minutes, maintaining the capsules in suspension.

9. Wash three times in saline.

10. Treat with 0.6% NaG for five minutes, maintaining the capsules in suspension.

0126537

11. Wash twice in saline.

12. Treat with 0.5% PVA in saline for five minutes maintaining the capsules in suspension.

13. Wash three times in saline.

5 14. Store capsules in saline or 2.5% albumin in saline to stabilize the insulin.

The capsules were suspended in saline and the extracapsular concentration of insulin was assayed daily. As shown in Figure 1, extracapsular concentration per 10 day ranged uniformly between about 3 and 4 units of insulin/ml of extracapsular saline. As shown in Figure 2, the cumulative insulin concentration in the extra-capsular medium rises at a substantially constant rate.

Example 2 - LIQUID INSULIN CAPSULES.

15 Blank gel spheres were made by dropping liquid spheres comprising 1.2% NaG in 0.9% saline into a 2.0% $CaCl_2/H_2O$ gelling solution. The $CaCl_2$ solution has a volume of 25 times that of the NaG solution. After formation, the gelled spheres were left in the $CaCl_2$ 20 for three minutes then treated as described below. All treatments and wash solutions had a volume of ten times the volume of the original NaG solution.

1. Wash with CHES buffer diluted 1:20 with 1.1% $CaCl_2$.

2. Wash with 1.1% $CaCl_2$.

25 3. Treat with 60,000 molecular weight poly-1-lysine (PLL) in saline (6.7mg PLL hydrobromide/dl) for four minutes, maintaining the capsules in suspension.

4. Wash with the CHES buffer-$CaCl_2$ solution as in step 1.

30 5. Treat with 0.11% polyvinylamine (PVA) in saline,

pH 7.0, for four minutes, maintaining the capsules in suspension.

6.   Wash three times with saline.

7.   Treat with 0.6% NaG in saline for four minutes, maintaining the capsules in suspension.

8.   Wash three times in saline.

This completes the formation of the blank micro-capsules. These capsules may be stored in saline until ready for loading and further treatment of the membranes.

The capsules were loaded with insulin by suspending them in an equal volume of 500 units insulin/ml for 15 to 18 hours at 37°C. As much as possible of the extra-capsular loading solution was then removed and the capsules were quickly washed once in four times their volume of saline. Immediately after washing the capsules were treated further using the procedure described below. All wash and treatment volumes were four times the volume of the capsules.

1.   Treat the capsules with 0.33% PVA in saline, pH 7.0, for six minutes keeping the capsules in suspension.

2.   Wash three times in saline.

3.   Treat with 0.06% NaG in saline for four minutes.

4.   Wash and store capsules in saline.

The finished capsules were suspended in saline containing 2.5% albumin. Extracapsular insulin concentration was measured daily. The results are illustrated in Figure 3. As shown, the cumulative insulin concentration increased at a substantially constant rate for a 5 day period.

0126537

Example 3 - MYOGLOBIN CAPSULES

Blank gel spheres were made by dropwise addition of a 1.2% sodium alginate solution in saline, to a 1.5% $CaCl_2/H_2O$ gelling solution. The $CaCl_2$ solution had a

5 volume of 25 times the volume of the NaG solution. After formation, the spheres were left in the $CaCl_2$ solution for four minutes and then treated as described below. All the solutions had a volume of ten times the volume of the original NaG solution.

10 1. Transfer to 0.6% $CaCl_2$ in 0.45% NaCl and allow to remain for five minutes.

2. Transfer to 0.3% $CaCl_2$ in 0.68% NaCl and allow to remain for five minutes.

3. Wash once in 0.9% saline.

15 4. Treat with 60,000 molecular weight poly-1-lysine (PLL) in saline (13.3mg PLL hydrobromide/dl) for five minutes, maintaining the capsules in suspension.

5. Wash with saline three times.

6. Wash with CHES buffer diluted 1:20 with 1.1%

20 $CaCl_2/H_2O$.

7. Treat with 0.17 polyvinylamine (PVA) in saline, pH 7.0, for five minutes, maintaining the capsules in suspension.

8. Wash three times with saline.

25 9. Treat with 0.6% NaG in saline for four minutes maintaining the capsules in suspension.

10. Wash two times in saline.

11. Treat with citrate solution (0.75% sodium citrate pH 7.4 in water).

30 12. Wash in 0.23% NaCl.

The blank capsules produced as disclosed above were loaded with myoglobin by suspending equal volumes of capsules and 2,000mg/dl of myoglobin in saline for 15 to 18 hours. The capsules were then washed with saline until the supernatant was clear, and then further treated using the procedure described below. All treatment and wash volumes were four times the volume of the capsules.

1.   Treat with 0.5% PVA in saline for five minutes maintaining the capsules in suspension.

2.   Wash three times in saline.

3.   Treat with 0.6% NaG in saline for four minutes meaintaining the capsules in suspension.

4.   Wash and store the capsules in 0.23% NaCl.

The capsules were then suspended in saline and the extracapsular solution was periodically assayed for myoglobin content. The results are shown in Figure 4. As illustrated, the cumulative myoglobin concentration increased at a substantially constant rate for nine days. These capsules released approximately 5-6mg myoglobin per dl per day.

Example 4 - HEMOGLOBIN LOADED CAPSULES.

Blank gelled spheres were made by dropwise addition of a 0.9% sodium alginate (Sigma) solution in water to a 2% $CaCl_2$ aqueous gelling solution. The $CaCl_2$ solution had a volume of 25 times the volume of the NaG solution. After formation, the spheres were left in the $CaCl_2$ solution for five minutes and then treated as described below. All treatment and wash solutions had a volume of ten times the volume of the original NaG solution.

0126537

1.   Wash with CHES buffer diluted with 1:20 with 1.1% $CaCl_2$.

2.   Treat with 40,000 molecular weight poly-l-lysine in 0.9% NaCl (6.7mg PLL hydrobromide/dl) for four minutes,

5   maintaining the capsules in suspension.

3.   Wash with the CHES buffer $CaCl_2$ as in step 1.

4.   Wash three times in 0.9% saline.

5.   Treat with 0.11% polyvinylamine (PVA) in saline pH 7.0 for four minutes, maintaining the capsules in

10   suspension.

6.   Wash three times in saline.

7.   Treat with 0.06% NaG in saline for four minutes, maintaining the capsules in suspension.

8.   Wash with saline three times.

15   9.   Treat with 0.75% trisodium citrate in 0.7% NaCl pH 7.4 for six minutes.

10.   Wash three times with saline.

The finished capsules are stored in saline until ready for loading and sealing.

20   The capsules were loaded with hemoglobin by suspending them in an equal volume of at least a 10% hemoglobin solution for 15 to 18 hours. To increase the internal hemoglobin solution was replaced and the above loading step was repeated. After loading, as much as

25   possible of the loading solution was removed and the capsules were washed quickly in saline until the supernate was nearly clear (a small amount of hemoglobin will slowly leak out of the capsules at this stage). Immediately after this wash, the capsules are further

30   treated using the procedures described below. All wash

and treatment volumes were four times the volume of the capsules.

1.   Treat the capsules with 0.17% PVA in saline, pH 7.0, for three minutes, maintaining the capsules in suspension.

2.   Wash three times in saline.

3.   Treat with 0.03% NaG in saline for four minutes.

4.   Wash three times and store the capsules in saline.

The hemoglobin capsules were suspended in saline and the extracapsular hemoglobin, concentration was assayed periodically. The results are set forth in Figure 5. As shown, the capsules released approximately equal daily quantities of hemoglobin and over a 14 day period.

The foregoing exemplary systems demonstrate that in accordance with the processes disclosed herein it is possible to produce compositions of matter which release at a substantially constant rate substances in the low, medium, and moderately high molecular weight range. Insulin has a molecular weight on the order of 6×10^3, myoglobin has a molecular weight on the order of 1.8×10^4, and hemoglobin has a molecular weight on the order of 7×10^4.

Since, as disclosed above, the capsule membranes can be made with physiologically compatible, non-toxic materials such as polypeptides and polysaccharides, the capsules produce no significant detectable immunlogical response when injected into, e.g. a body cavity, under the skin, or into muscle tissue of an animal. Accordingly, various biologically active materials may be

0126537

administered to animals at a substantially constant rate
by implanting, preferably by injection, one or more
capsules of the type described above. A substantially
constant rate of release can be achieved for a significant
5   period of time provided that the extracapsular concen-
tration of the substance immediately adjacent the capsules
does not build up to a level sufficient to alter the
kinetics of release.

The compositions can be stored by suspending the
10   capsules in a minimum volume of a solution of the
substance contained in the intracapsular volume. Water-
containing capsules may be suspended in a lipophilic
vehicle to achieve the same purpose.

The invention may be embodied in other specific
15   forms without departing from the spirit and scope
thereof.

CLAIMS

1.   A dispensing system for releasing a substance at a substantially constant rate in an environment that depletes said substance, said system comprising:

5   a capsule having a membrane which defines an intracapsular volume, said membrane having a multiplicity of pores of dimensions sufficient to limit the rate of which the molecules of said substance pass therethrough from said intracapsular volume; and

10   disposed within said volume, a liquid reservoir of said substance at a concentration of said substance which results in an osmotic pressure sufficient to sustain said limited rate when said capsule is placed in said environment.

15 2.   The system of claim 1 for purposes of maintaining a given extracapsular concentration in said environment wherein the reservoir of said substance within said intracapsular volume is at a concentration at least two orders of magnitude greater than said given concentration.

20 3.   The system of claim 1 wherein said substance is a solid disposed within said intracapsular volume in a solvent for said solid.

4.   The system of claim 3 wherein said solid is
25 sparingly soluble in said solvent.

5.   The system of claim 1 wherein said substance comprises a biologically active material.

6.   The system of claim 5 wherein said biologically active material is selected from the group consisting of hormones, enzymes, antibodies, vaccines, drugs, and lymphokines.

7.   The system of claim 1 wherein said substance is selected from the group consisting of plant fertilizers, pesticides, fungicides, and plant hormones.

8.   The system of claim 1 wherein said substance is selected from the group consisting of flavors, perfumes, and preservatives.

9.   The system of claim 1 wherein said substance is a tissue culture nutrient.

10.   The system of claim 1 further comprising, for storage purposes, an extracapsular volume of said substance at a concentration sufficient to substantially prohibit a net flow of said substance from said intracapsular volume to said extracapsular volume.

11.   The system of claim 1 wherein said membrane comprises a matrix of polycationic polymer salt bonded to a polyanionic polymer.

12.　The system of claim 1 wherein said membrane defines a sphere having a diameter between about 0.1 and 3.0 mm.

5　13.　A process for producing the dispensing system of claim 1 comprising the steps of:-

A.　forming a capsule which defines an intracapsular volume and comprises a membrane defining a multiplicity of pores of a size sufficient to permit passage of molecules of said substance;

10　B.　placing said capsule in a solution containing a concentration of said substance for a time sufficient to permit said solution and said substance to diffuse into said intracapsular volume to produce a capsule loaded with said substance; and

15　C.　reducing the dimensions of the pores defined by the membrane of said loaded capsule to a degree sufficient to limit the rate at which molecules of said substance pass therethrough.

14.　The process of claim 13 wherein step A comprises

20　the steps of:-

1)　forming a shape-retaining sphere comprising a water-soluble polymer comprising plural cationic or anionic groups; and

2)　contacting said sphere with a first polymer

25　comprising plural groups having a charge opposite that of said water-soluble polymer to produce a capsule comprising a porous membrane about said sphere.

15.   The process of claim 13 wherein, in step A, the
dimensions of said pores are controlled such that passage
of the molecules of said substance is hindered and in
step B, the rate of diffusion of said substance into
5   said capsule is controlled by said membrane.

16.   A method of producing a system for releasing a
substance at a predetermined rate comprising forming a
permeable capsule, suspending the capsule in a solution
containing the substance thereby to load the substance
10   into the capsule, and post-treating the capsule to reduce
its permeability.
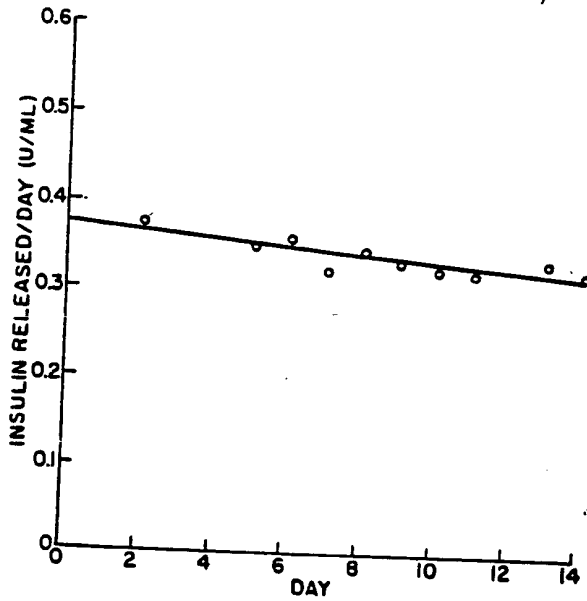
FIG. 1
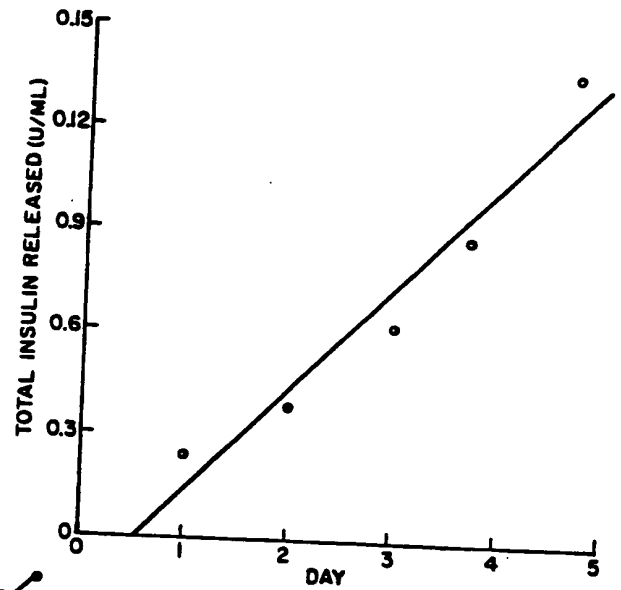
FIG. 2
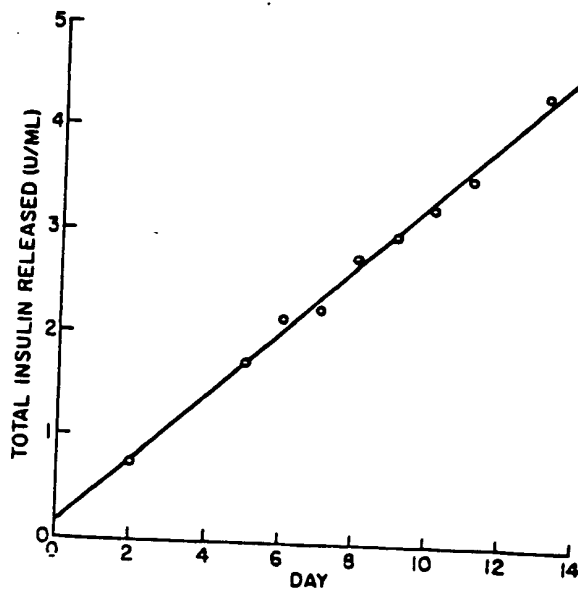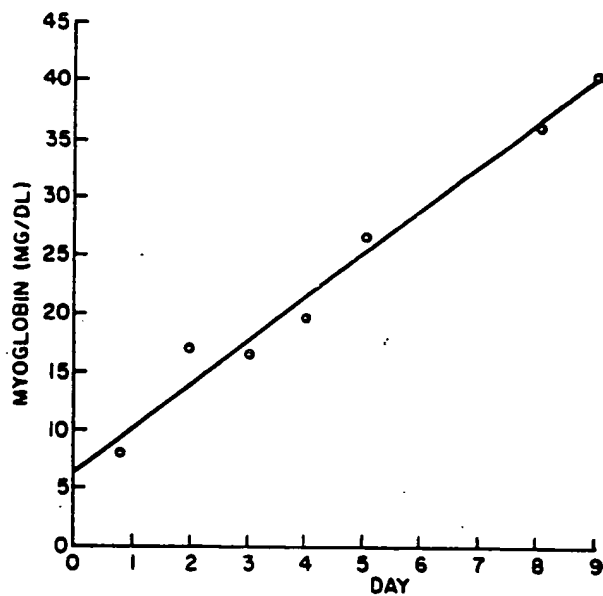

FIG. 3

FIG. 4
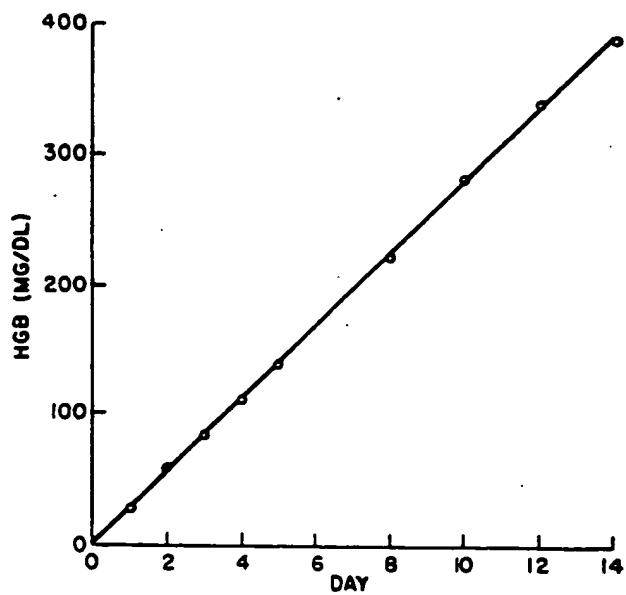
FIG. 5

This Page Blank (uspto)